Difference in volatiles of poplar induced by various damages

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Abstract: Three treatments including mechanical damage, *Lymantria dispar* attacking and daubing oral secretions of the insects on mechanically damaged cut were conducted on *Populus simonii×Populus pyramibalis* c.v. in order to find the genuine reason leading to effective resistance response of tree to insects attacking. The release situation of the induced volatiles of the plant was analyzed by TCT-GC/MS at 24 hours after damages. The results indicated that some of the volatiles such as (Z)-3-hexenyl acetate, decanal, 3-hexenyl isovalerate, nonanal, ocimene, and 2-cyanobutane can be induced by both insects attacking and mechanical damage, while 2,6-dimethyl-1,3,5,7-octatetraene, 2-methyl-6-methylene-1,7-octadien-3-one, caryophyllene, Isovaleronitrile, diethyl-methyl-benzamide, and dicapryl phthalate were only induced by insects attacking. Such difference in volatiles was attributed to that there existed active components in oral sections of the larvae of *Lymantria dispar*

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Introduction

Plants can emit volatile compounds through stomas in response to wounding, which are called wound-induced volatiles, and these compounds play critical roles in the trophic triangle of plant-herbivore-natural enemy. (Kessler *et al.* 2001a, 2002b; Halitschke *et al.* 2001). Wound-induced volatiles are important for herbivores to choose plant host. They can exclude the same herbivore species, attract others, and act as pheromone to attract the predators. Furthermore, as alarm signals between plants, wound-induced volatiles transmit wounding signals to healthy plants nearby, leading to resistance reaction in advance (Kahl *et al.* 2000; Turlings *et al.* 1998; Schittko *et al.* 2000).

The quantities of volatiles for cotton induced by mechanical damage are less than that induced by caterpillar feeding (Paré *et al.* 1999). In undamaged leaves of lima bean closing to the leaves attacked by herbivores, five defensive genes are induced. While leaves close to the mechanically damaged leave, the genes will not be expressed (Wang *et al.* 1999). All above results showed that there were differences in the mechanisms of volatiles induced by mechanical damage and insects attacking. In the research of insects attacking cotton, the inducement of volatiles released from cotton is an active process, which needs to be renewedly synthesized. The inducible signal compounds exist in the oral secretions of insects (Loughrin *et al.* 1995; Mattiacci *et al.* 1995).

In this paper, the induced volatiles by mechanical damage and daubing oral secretions of the larvae of *Lymantria dispar* from the *Populus simonii×Populus pyramibalis* c.v were investigated in order to find the genuine reason leading to effective resistance response to insects attacking.

Materials and methods

Materials

The cuttings of *P. simonii×P. pyramibalis* c.v were transplanted to plastic pots in March. The holes at the bottom of pots were obstructed to avoid the roots growing out of the pots and resulting in damages of the root systems. The seedlings were watered daily and irrigated with nutritive solution (Hoagland) each month before the experiment. The third instar larvae of *Lymantria dispar* were provided by Insect Institute of Xiangshan Forestry Center.

Wounding treatments and volatiles collection

Mechanical damage: Intact seedlings were chosen and cut off 20% of leaf area with scissors.

Insects attacking: First, 8 to 10 healthy larvae of *Lymantria dispar* were put on to each plant, and then they were taken down when 20% of leaf area was damaged.

Daubing oral secretions of the insects on mechanically damaged cut: The secretions extracted by capillary from the mouths of healthy insects were daubed on the cut of the seedlings, where about 20% of the leaf area was cut off.

Volatiles collection: After the 22.5 hours of the wounding treatment, the Renolds Oven Bag (44.3 cm × 55.8 cm, which gave out few volatiles under high temperature and

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light intensity) was put on the plant. A glass tube (15 cm×0.3 cm) filled with 200 mg adsorbent-Tanex-TA was used to collect the volatiles. The glass tube and the seedling were untouched. As shown in Fig.1, the air in the bag was quickly extracted, and then the clean air which was filtered through activated carbon and absorbent- GDX-101 was pumped into the envelope for 30 min. Afterwards, the volatiles were collected for an hour at a flow rate of 90 mL·min^{-1}.

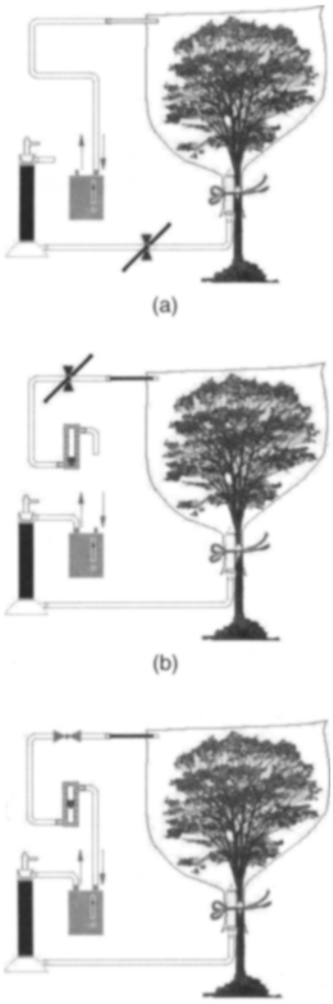

Fig.1 Volatiles collection.
(a) Pumping off air from bag. (b) Pumping filtered air into bag. (c) Capturing volatiles within circulation system.

Analysis and identification of volatiles

The TCT-GC/MS method was used to identify the volatiles.

TCT working condition

System pressure: 20 KPa; rod temperature: 250 °C; trap precool: -120 °C; tube desorption: 250 °C (10 min); trap inject: 260 °C (1 min).

GC working condition

Chromatogram columniation: CP-Sil8 Low; Bleed/MS (60 m×0.25 mm×0.25 μm); programmed temperature: from 40°C (3 min) to 250°C (3 min) at 6°C /min; post run 270°C (5 min).

MS working condition

Ionization mode: EI; electronic energy: 70 eV; mass range: 29–350 amu; scan speed: 0.4 sec·scan^{-1}; interfacial temperature: 250°C; temperature of ion source: 200°C; mission current: 150 μA.

Analytic software

The Xcalibur software system provided by Finnigan was used in the analysis.

Quantitative method of the volatiles

Peak area normalization was applied to obtain the content of every volatile.

Results

In the experiment, we found that all the three wounding treatments through TCT-GC/MS can induce the emission of volatiles from the seedlings (Table 1). Some of the volatiles, such as (Z)-3-hexenyl acetate, decanal, 3-hexenyl isovalerate, nonanal ocimene, and 2-cyanobutane, can be induced by not only insects attacking but also mechanical damage, while some others were triggered only by insects attacking. These results indicated that the mechanisms of inducing volatiles by insects attacking and mechanical damage were different. The volatiles species induced by daubing oral secretions of the insect were similar to those resulting from insects attacking directly. Some of those can not be induced by mechanical damage, such as 2, 6-dimethyl-1, 3, 5, 7-octatetraene, caryophyllene, isovaleronitrile. Moreover, though some volatiles can be induced by all the three wounding treatments, for example, nonanal, decanal, and 2-cyanobutane, their contents by insects attacking and daubing oral secretions on mechanically damaged cut were much higher than those by the mechanical damage, which showed the oral secretions of the insects magnified the emission of volatiles.

Discussion

The insects attacking can induce special volatiles which are different from those by mechanical damages. These volatiles were the main factor in natural enemy of pest searching for the host. They might play a crucial role in the trophic triangle of plant-pest-natural enemy (Thomas *et al.* 1999; Ziegler *et al.* 2001). The discrepancy of the volatiles between insects attacking and mechanical damage indicated that the oral secretions served as a key role.

In this paper, there were two factors involved in the release of volatiles by insects attacking. One was the influ-

ence of the mechanical damage caused by insects chewing, and the other which was more important was the oral secretion, for it contains some active components. The mechanical damage destroyed the plant cells, and then some volatile compounds stored in vacuole gave out. Moreover, mechanical damage can activate some signal transduction pathways to induce volatiles, such as (Z)-3-hexenyl acetate, decanal. The oral secretions of insects played an important role in the emission of induced volatiles when plants were infected by insects. When insects attacked plants besides mechanical damage, the effective components in the oral secretions of insect bonded with their receptors on the membrane of the plant cell, then some special signal cascades were activated and more volatiles gave out, such as caryophyllene, dicapryl phthalate etc. A compound *N*-(17-hydroxylinolenoyl)–L- glutamine (named volicitin) had been isolated from oral secretions of beet armyworm caterpillars (Alborn *et al.* 1997). When volicitin was applied to damage leaves of corn seedlings, it induced the seedlings to emit volatile compounds that attract parasitic wasps, natural enemies of the caterpillars and mechanical damage of the leaves, without application of this compound, the same blend of volatiles were not triggered to emit.

Table 1. Contents of some volatiles from seedling after three treatments

Treatments	Contents of the volatiles (%)					
	2,6-dimethyl-1,3,5,7-octatetraene	(Z)-3-hexenyl acetate	2-methyl-6-methylene-1,7-octadien-3-one	Decanal	3-hexenyl isovalerate	Nonanal
Mechanical damage		2.49		0.37	0.41	1.21
Insect attacking	1.32	6	2.33	1.3	0.14	10
Daubing oral secretions of the insect on mechanically damaged cut	0.25	7.9	12.11	1.84	0.28	10.19

Treatments	Contents of the volatiles (%)					
	Ocimene	Caryophyllene	2-cyanobutane	Lsovaleronitrile	Diethyl-methyl-benzamide	Dicapryl phthalate
Mechanical damage	0.03		0.13			
Insect attacking	0.38	0.19	0.94	0.37	0.45	8.94
Daubing oral secretions of the insect on mechanically damaged cut	0.07	0.86	0.88		0.11	1.9

Note: The numbers in the table indicate the percent content of different volatiles in the total ones.

Furthermore, plants can synthesize and emit volatiles in response to diverse herbivore species owing to the difference of the oral secretions (De Moraes *et al.* 1998), thus it was necessary to identify the effective components in the oral secretions of insect in the further research.

References

Alborn, H.T., Turlings, T.C.J., Jone, T.H., *et al.* 1997. An elicitor of plant volatiles from beet armyworm oral secretion [J]. Science, **276**: 945–949.

De Moraes, C.M., Lewis, W.J., Paré, P.W., Alborn, H.T., *et al.* 1998. Herbivore-infested plants selectively attract parasitoids [J]. Nature, **393**: 570–573.

Halitschke, R., Schittko, U., Pohnert, G., *et al.* 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses [J]. Plant Physiol, **125**: 711–717.

Kahl, J., Siemens, D.H., Aerts, R.J., *et al.* 2000. Herbivore -induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore [J]. Planta, **210**: 336–342.

Kessler, A., Baldwin, I.T. 2001a. Defensive function of herbivore-induced plant volatile emissions in nature [J]. Science, **291**(16): 2141–2144.

Kessler, A., Baldwin, I.T. 2002b. Plant-mediated tritrophic interactions and biological pest control [J]. AgBiotechNet, **4**: 1–7.

Koch, T., Krumn, T., Jung, V., *et al.* 1999. Differential induction of plant volatile biosynthesis in the lima bean by early and late intermediates of the octadecanoid-signaling pathway [J]. Plant Physiol, **121**: 153–162.

Loughrin, J.H., Potter, D.A., Hamilton-Kemp, T.R., *et al.* 1995. Volatile compounds induced by herbivores act as aggregation kairom ones for the Japanese beetle (*Popillia japonica* Newmen) [J]. J. chem. Ecol., **21**(10): 1457–1467.

Mattiacci, L., Dicke, M., Posthumus, M.A. 1995. β-glucosidase:An elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps [C]. Proc Natl Acad Sci USA., p2036–2040.

Paré, P.W. and Tumlinson, J.H. 1999. Plant volatiles as a defense against insect herbivores [J]. Plant Physiol, **121**: 325–331.

Schittko, U., Preston, C.A., Baldwin, I.T. 2000. Eating the evidence? *Manduca sexta* can not disrupt specific jasmonate induction in *Nicotiana attenuata* by rapid consumption [J]. Planta, **210**: 343–346.

Wang Shaofang, Emilio, L.G., Smith, J.R. 1999. Volatiles from trifolium as feeding deterrents of redlegged earth mites [J]. Phytochemistry, 52: 601–605.

Turlings, T.C.J., Lengwiler, U.B., Bernasconi, M.L., *et al.* 1998. Timing of induced volatile emissions in maize seedlings [J]. Planta, **207**:146–152.

Ziegler, J., Keinänen, M., Baldwin, I.T. 2001. Herbivore induced allene oxide synthase transcripts and jasmonic acid in *Nicotiana attenuate* [J]. Phytochemistry, **58**: 729–738.